

Listing of the Claims

What is claimed is:

1. (currently amended) A method for DNA synthesis at high pH, comprising: a) ~~providing a DNA polymerase fusion; and~~ contacting said a DNA polymerase fusion with a nucleic acid template under conditions of high pH, and b) effecting template dependent synthesis of DNA, wherein said fusion permits DNA synthesis wherein said high pH ranges from 9.3 to 14, and wherein said DNA polymerase fusion comprises wild type *Pyrococcus furiosus* polymerase I fused to *Sulfolobus solfataricus* SSso7d protein, wherein said DNA polymerase fusion functions as a DNA polymerase.
2. (currently amended) The method of claim 1, further comprising contacting a PCR enhancing factor and/or an additive with said DNA polymerase fusion and said nucleic acid template.
3. (currently amended) A method for the cloning of a DNA synthesis product, at high pH, wherein said high pH ranges from 9.3 to 14, comprising:
 - a) providing a DNA polymerase fusion comprising wild-type *Pyrococcus furiosus* polymerase I fused to *Sulfolobus solfataricus* SSso7d protein, wherein said DNA polymerase fusion functions as a DNA polymerase;
 - b) contacting said DNA polymerase fusion with a nucleic acid template, under conditions of said high pH so as to effect the template dependent synthesis of a DNA synthesis product, wherein said fusion permits DNA synthesis to generate a synthesized DNA product; and
 - c) inserting said synthesized DNA product into a cloning vector, thereby cloning said synthesized DNA product .
4. (currently amended) The method of claim 3, further comprising contacting a PCR enhancing factor and/or an additive with said DNA polymerase fusion and said nucleic acid template in step (b).
5. (currently amended) A method for sequencing DNA at high pH, wherein said high pH ranges from 9.3 to 14, comprising the steps of:
 - (a) contacting a template DNA strand with a sequencing DNA primer;

- (b) contacting said DNA of step (a) with a DNA polymerase fusion comprising wild-type *Pyrococcus furiosus* polymerase I fused to *Sulfolobus solfataricus* SSso7d protein, wherein said DNA polymerase fusion functions as a DNA polymerase, deoxyribonucleoside triphosphates, and a chain-terminating nucleotide analog;
 - (c) incubating the mixture of step (b) under conditions sufficient to synthesize at said high pH a random population of DNA molecules complementary to said ~~first~~ DNA molecule, wherein said synthesized DNA molecules are shorter in length than said first DNA molecule and wherein said synthesized DNA molecules comprise a terminator nucleotide at their 5' termini; and
 - (d) separating said synthesized DNA molecules by size so that at least a part of the nucleotide sequence of said first DNA molecule can be determined.
6. (Original) The method of claim 5, further comprising a PCR enhancing factor and/or an additive.
7. (Currently amended) A method of linear or exponential PCR amplification at high pH, wherein said high pH ranges from 9.3 to 14, for site-directed or random mutagenesis comprising the ~~step~~ steps of: incubating a reaction mixture comprising a nucleic acid template, at least one PCR primers, and a DNA polymerase fusion comprising wild-type *Pyrococcus furiosus* polymerase I fused to *Sulfolobus solfataricus* SSso7d protein, wherein said DNA polymerase fusion functions as a DNA polymerase, under conditions which permit amplification of said nucleic acid template at said high pH by said fusion to produce a mutated amplified product.
8. (Original) The method of claim 7, further comprising a PCR enhancing factor and/or an additive.
9. (currently amended) A method of reverse transcriptase PCR at high pH, wherein said high pH ranges from 9.3 to 14, comprising the ~~step~~ steps of incubating a reaction mixture comprising a nucleic acid template, at least one PCR primer, and a DNA polymerase fusion comprising wild-type *Pyrococcus furiosus* polymerase I fused to *Sulfolobus solfataricus* SSso7d protein, wherein said DNA polymerase fusion functions as a DNA polymerase, under reaction conditions which permit amplification of said nucleic acid template at said high pH by said fusion to produce an amplified product.

10. (Original) The method of claim 9, further comprising a PCR enhancing factor and/or an additive.
11. (currently amended) The method of claim 1, 3, 5, 7 or 9, wherein said DNA polymerase fusion has reduced DNA polymerization activity relative to wild-type *Pyrococcus furiosus* polymerase I under identical reaction conditions.
12. (withdrawn) The method of claim 11, wherein said DNA polymerase fusion comprises a Glycine to Proline substitution at amino acid position 387 (G387P) and has reduced DNA polymerization activity.
13. (currently amended) The method of claim 1, 3, 5, 7 or 9, wherein said DNA polymerase fusion comprises reduced base analog detection activity relative to wild-type *Pyrococcus furiosus* polymerase I under identical reaction conditions.
14. (withdrawn) The method of claim 13, wherein said DNA polymerase fusion comprises reduced base analog detection activity and a mutation at position V93, wherein said mutation is a Valine to Arginine substitution, a Valine to Glutamic acid substitution, a Valine to Lysine substitution, a Valine to Aspartic acid substitution or a Valine to Asparagine substitution.
15. (Currently amended) The method of claim 11 wherein said DNA polymerase fusion has reduced base analog detection activity relative to wild-type *Pyrococcus furiosus* polymerase I under identical reaction conditions.
16. (withdrawn) (Currently amended) The method of claim 12, wherein said DNA polymerase fusion comprises reduced base analog detection activity relative to wild-type *Pyrococcus furiosus* polymerase I under identical reaction conditions.
17. (withdrawn) The method of claim 11, wherein said DNA polymerase fusion further comprises a mutation at position V93, wherein said mutation is a Valine to Arginine substitution, a Valine to Glutamic acid substitution, a Valine to Lysine substitution, a Valine to Aspartic acid substitution or a Valine to Asparagine substitution that confers a reduced base analog detection activity phenotype to said chimeric DNA polymerase.
18. (withdrawn) The method of claim 12, wherein said DNA polymerase fusion further comprises a mutation at position V93, wherein said mutation is a Valine to Arginine substitution, a Valine to Glutamic acid substitution, a Valine to Lysine substitution, a

Valine to Aspartic acid substitution or a Valine to Asparagine substitution that confers a reduced base analog detection activity phenotype to said chimeric DNA polymerase.

19. (Currently amended) The method of claim 13, wherein said DNA polymerase fusion further comprises a reduced DNA polymerization activity relative to wild-type *Pyrococcus furiosus* polymerase I under identical reaction conditions.
20. (withdrawn) (Currently amended) The method of claim 14, wherein said DNA polymerase fusion further comprises a reduced DNA polymerization relative to wild-type *Pyrococcus furiosus* polymerase I under identical reaction conditions.
21. (withdrawn) The method of claim 13, wherein said DNA polymerase fusion further comprises a Glycine to Proline substitution at amino acid position 387 (G387P) that confers a reduced DNA polymerization phenotype to said chimeric DNA polymerase.
22. (withdrawn) The method of claim 14, wherein said DNA polymerase fusion further comprises a Glycine to Proline substitution at amino acid position 387 (G387P) that confers a reduced DNA polymerization phenotype to said chimeric DNA polymerase.
23. (withdrawn) The method of claim 1, 3, 5, 7 or 9, wherein said DNA polymerase fusion further comprises an Aspartate to alanine substitution at amino acid 141 (D141A) and a Glutamic acid to Alanine substitution at amino acid position 143 (D141A/E143A) that renders said chimeric DNA polymerase 3'-5' exonuclease deficient.
24. (withdrawn) The method of claim 13, wherein said DNA polymerase fusion with reduced base analog detection activity further comprises an Aspartate to alanine substitution at amino acid 141 (D141A) and a Glutamic acid to Alanine substitution at amino acid position 143 (D141A/E143A) that renders said chimeric DNA polymerase 3'-5' exonuclease deficient.
25. (original) The method of claim 1, 3, 5, 7 or 9, wherein said DNA polymerase fusion comprises a wild type, mutant or chemically modified DNA polymerase.
26. (currently amended) The method of claim 1, 3, 5, 7, or 9, wherein said DNA polymerase fusion is a proofreading polymerase.

27. (currently amended) The method of claim 26, wherein said proofreading polymerase ~~is selected from the group consisting of~~ comprises wild-type *Pyrococcus furiosus* polymerase I Pfu, KOD, Tgo, Vent and DeepVent.
28. (original) The method of claim 1, 3, 5, 7 or 9, wherein said DNA polymerase fusion further comprises a polypeptide with at least one of an increase in an activity selected from the group consisting of: processivity, proofreading, fidelity, DNA binding activity, strand displacement activity, polymerase activity, nucleotide binding and recognition, efficiency, template length amplification capability, GC-rich target amplification efficiency, specificity, thermostability, intrinsic hot start capability, or salt resistance.
29. (original) The method of claim 1, 3, 5, 7 or 9, wherein said DNA polymerase fusion further comprises a polypeptide with at least one of a reduced activity selected from the group consisting of: DNA polymerase activity at room temperature, amplification slippage on templates with tri-nucleotide repeat stretches, extension time in a PCR reaction or amplification cycles in a PCR reaction.
30. (currently amended) The method of claim 1, 3, 5, 7 or 9, wherein said DNA polymerase fusion ~~consists of~~ comprises a DNA binding protein domain, wherein said DNA binding protein domain is selected from the group of: thioredoxin processivity factor binding domain of bacteriophage T7, archaeal PCNA binding domain, PCNA, the helix hairpin helix DNA binding motifs from DNA topoisomerase V or the DNA binding protein *Sulfolobus solfataricus* SSso7d protein Sso7d or Sae7d.
31. (withdrawn)(currently amended) A kit for performing at high pH, wherein said high pH ranges from 9.3 to 14, a method selected from the group consisting of: DNA synthesis; cloning of a DNA synthesis product; sequencing DNA; RT PCR; and linear or exponential PCR amplification comprising a DNA polymerase fusion and packaging materials therefore.
32. (withdrawn)(currently amended) The kit of claim 31, further comprising a high pH buffer, wherein said high pH buffer has a pH which ranges from 9.3 to 14.
33. (withdrawn) The kit of claim 31, further comprising a PCR enhancing factor and/or an additive.

34. (withdrawn)(currently amended) A composition for any one of DNA synthesis, cloning of a DNA synthesis product at high pH, sequencing DNA, linear or exponential PCR amplification for site directed or random mutagenesis, RT-PCR comprising a DNA polymerase fusion and a high pH buffer, wherein said high pH buffer has a pH which ranges from 9.3 to 14.
35. (withdrawn)(currently amended) A composition for DNA synthesis, comprising a DNA polymerase fusion and a high pH DNA synthesis buffer, wherein said high pH DNA synthesis buffer has a pH which ranges from 9.3 to 14.
36. (withdrawn)(currently amended) A composition for cloning of a DNA synthesis product, comprising a DNA polymerase fusion and a high pH DNA cloning buffer, wherein said high pH cloning buffer has a pH which ranges from 9.3 to 14.
37. (withdrawn)(currently amended) A composition for sequencing DNA, comprising a DNA polymerase fusion and a high pH DNA sequencing buffer, wherein said high pH sequencing buffer has a pH which ranges from 9.3 to 14.
38. (withdrawn)(currently amended) A composition for linear or exponential PCR amplification for site directed or random mutagenesis, or for RT-PCR comprising a DNA polymerase fusion and a high pH PCR reaction buffer, wherein said high pH PCR reaction buffer has a pH which ranges from 9.3 to 14.
39. (withdrawn) The composition of claims 34, 35, 36, 37 or 38, further comprising a PCR enhancing factor and/or an additive.
40. (New) The method of any one of claims 1, 3, 5, 7, and 9, wherein said DNA polymerase fusion is encoded by SEQ ID NO: 126 and has an amino acid sequence of SEQ ID NO:127.